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Response of Lesser Grain Borer, *Rhizopertha dominica* (Fabr.) [Coleoptera: Bostrichidae] to Powders and Extracts of *Azadirachta indica* and *Piper guineense* Seeds

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Abstract

The powders and extracts of *Azadirachta indica* A. Juss and *Piper guineense* (Schum and Thonn) seeds were bioassayed for their insecticidal potential against the lesser grain borer, *Rhizopertha dominica* (Fabr.) in the laboratory at 30±2^{0}C and 70±5% relative humidity. The powders were applied at rates 0.5, 1.0, 2.0 and 4.0g/20g of wheat grains. The extracts of the plants were also applied at rates 1, 2, 3 and 4%/20g of wheat grains. The parameter studies were adult mortality, adult emergence, % reduction of adult emergence and weight loss in treated wheat grains. The results obtained shows that adult mortality of *R. dominica* increased as concentration of powders and extracts increased. The two plants powders were able to evoked 100% mortality of adult *R. dominica* at rates 1.0, 2.0 and 4.0g/20g of wheat grains after 72 hours of post treatment. Adult emergence shows that more adult emerged from the control (46) which was significantly higher (P<0.05) than others. The extracts from the two plants seeds tested against *R. dominica* were able to caused 100% mortality of lesser grain borer within 48 hours of post treatment at all tested concentrations. There was no adult emergence of *R. dominica* in wheat treated with extracts compared to control (treated with solvent) which recorded 19 adult emergence and control (without solvent) which had 43 adult emergence. The extracts did not affect the weight of treated wheat grains. The results obtained from this study justified that powders and extracts of *A. indica* and *P. guineense* seeds can be used as biopesticides against *R. dominica*. The two plants are of medicinal values, biodegradable, readily available and poses no danger to man and other mammals.

Keywords: Adult emergence and biopesticides; *Rhyzopertha dominica*; *Azadirachta indica*; *Piper guineense*

1. Introduction

Wheat is a cereal grain of the monocot plant *Triticum* spp (Belderok *et al*., 2000) and it is the world's most important cereal crop in relation to production and consumption (Ileke, 2011).

Wheat is grown on more land area than any other commercial crop. It is the most important staple food for humans. World trade in wheat is greater than all other crops combined (Curtis and Macpherson, 2002). Wheat is the leading source of protein in human foods, having a higher protein content than either maize or rice, the other major cereal grains. In terms of total production tonnages used for food, it is currently second to rice as the main human food crop and ahead of maize. As a cereal grains, it is the most proteinous grains consumed in developing countries to combat malnutrition in young children, especially in Nigeria (Ileke, 2011)

Wheat is attacked by various insect pests between harvest and storage. The most economically important insect pests of stored wheat are the granary weevils, *Sitophilus granarius*, maize weevils, *Sitophilus zeamais*, rice weevils, *Sitophilus oryzae*, lesser grain borer, *Rhizopertha dominica*, larger grain borer, *Prostephanus truncatus*, Angoumois grain moth, *Sitotroga cerealella,* Indian meal moth, *Plodia interpunctella*, rice moth, *Corcyra cephalonica* and red flour beetle, *Tribolium castaneum* (Adedire, 2001; Ileke, 2011). Lesser grain borer, *R. dominica* is a field-to-store pest and this may cause economic damage in the store (Adedire, 2001).

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Presently, insect pests control in stored food products relies on the use of synthetic insecticides which has some hazards such as pollution of the environment, toxic residues on stored grains, development of resistance by target species, pest resurgence and lethal effects on non-target organisms in addition to direct toxicity to users and health hazard (Adedire and Lajide, 2003; Adedire *et al*., 2011; Ileke and Oni, 2011, Udo, 2011; Ileke and Olotuah, 2012; Ileke and Bulus, 2012). Recently, there is a steady increase in the use of medicinal plant products as a cheaper and ecologically safer means of protecting stored products against infestation by insects (lvbijaro and Agbaje, 1986; Adedire and Ajayi, 1996; Adedire and Lajide, 2003; Ashamo and Odeyemi, 2001; Oni and Ileke, 2008; Adedire *et al*., 2011; Akinkurolere *et al*., 2006; 2009; Ileke *et al*., 2012) and the tropics is well endowed with these plant species some of which are also used for medicinal purposes (Adedire and Lajide, 2003; Ileke, 2008). Vegetable oils, plant powders and extracts have been used to reduce post harvest losses of cereals and grain legumes (Odeyemi, 1998; Adedire and Lajide, 1999; Ofuya *et al*., 2007; Nwaubani and Fasoranti, 2008). Therefore, this present study investigated the response of *R. dominica* to powders and extracts of *Azadirachta indica* and *Piper guineense* seeds in stored wheat grains. These plants have been investigated to be effective in protecting cowpea and maize seeds from infestation by cowpea bruchid, *Callosobruchus maculatus* and *Sitophilus zeamais* respectively (Lale and Abdulrahman, 1999; Adedire and Lajide, 2003; Asawalam *et al*., 2007; Ileke and Oni, 2011; Ileke and Bulus, 2012).

2. Materials and Methods

2.1. Insect culture

Adult lesser grain borer, *R. dominica* used for this study were obtained from an already existing culture in the Environmental Biology and Fisheries Research Laboratory, Adekunle Ajasin University, Akungba Akoko, Nigeria. They were reared on disinfested wheat grains, *T. aestivum* variety hard red winter collected from a seed warehouse in Akure, Ondo State, Nigeria. The grains were cleaned of foreign matter and disinfested by keeping in freezer at -5^{o}C for 7 days. This is because all the life stages, particularly the eggs are very sensitive to cold. The disinfested grains were then air dried in the laboratory to prevent mould growth (Adedire *et al*., 2011) before introduction of insects. They were placed in Kilner jars and covered with muslin cloth. The jars were placed in insect rearing cages at ambient temperature of 30$\pm$2^{o}C and 75$\pm$5% relative humidity.

Wheat, *T. aestivum* grains used for the experiment were also disinfested as described above before it was stored in plastic containers with tight lids disinfested by swabbing with absolute ethanol.

2.2. Collection of plant materials and preparation of extracts

The plant materials used in this study were *Azadirachta indica and Piper guineense* seeds. These materials were sourced fresh from Akola farm at Igbara-Odo Ekiti, Ekiti State, Nigeria. The seeds were sun dried for 3 days before air dried in the laboratory. The cleaned dried seeds were pulverised into fine powders using a blender. The powder were further sieved to pass through 1mm^2 mesh (Ileke and Bulus, 2012). The powders were packed in plastic containers with tight lids and kept in the dark (Udo, 2011). Acetone extracts of *A. indica* and *P. guineense* seeds powders were carried out using cold extraction method. About 150g of *A. indica* and *P. guineense* powders were soaked separately in an extraction bottle containing 100% acetone. The mixture was stirred occasionally with a glass rod and extraction was terminated after 72 hours. Filtration was carried out using a double layer of Whatman No. 1 filter papers and acetone evaporated using a rotary evaporator at 30 to 40^{o}C with rotary speed of 3 to 6 rpm for 8 hours (Udo, 2011; Ileke and Olotuah, 2012). The resulting extract was air dried in order to remove traces of solvent. The crude extract obtained was stored in the refrigerator prior to use (Aina *et al*., 2009). From this stock solution, different extract concentration of 1%, 2%, 3% and 4% were prepared as follows: 1% concentration was made by diluting 0.1ml of extract in 9.9ml of acetone; 2% concentration was made by diluting 0.2ml of extract in 9.8ml of acetone; 3% concentration was made by diluting 0.3ml of extract in 9.7ml of acetone, Similarly, 4% concentration was made by diluting 0.4ml of extract in 9.6ml of acetone (Ashamo and Akinnawonu, 2012).

2.3. Toxicity of plant powders on mortality and adult emergence of R. dominica

The plant powders where thoroughly mixed with 20g of wheat grains in 250ml plastic containers at 0.0 (untreated), 0.5, 1.0, 2.0 and 4.0g corresponding to 2.5, 5, 10 and 20% w/w concentration (Fatope *et al*., 1995). The containers with their contents were gently shaken to ensure thorough admixture of the wheat grains and treatment powders. Twenty newly emerged adults *R. dominica* were randomly (unsexed) picked and introduced to each of the containers and covered. Four replicates of the treatments and untreated controls were laid out in Complete Randomized Design. Beetle mortality was observed daily for 4 days. After every 24 hours, the number of dead beetles were counted and recorded. The beetles were confirmed dead when there was no response to probing with sharp pin at the abdomen (Adedire *et al*., 2011). At the end of day 4, all insects, both dead and alive were removed from each container. The experiment was kept inside the insect cage for another 30 days to allow for the emergence of the first filial (F1) generation. The number of adults that emerged from each replicate was counted and recorded. The percentage reduction in adult emergence of F_1 progeny or inhibition rate (IR) was calculated according to the method described by Tapondju *et al*. (2002).

$$\% IR = \frac{C_n - T_n}{C_n} \times \frac{100}{1}$$

where C_n is the number of emerged insects in the control and T_n is the number of emerged insects in the treated containers.

The % loss in weight was determined and recorded using the method described by Odeyemi and Daramola (2000).

$$\% \text{ Weight loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{initial weight}} \times \frac{100}{1}$$

2.4. Toxicity of acetone extracts on mortality and adult emergence of R. dominica

Extracts of *A. indica* and *P. guineense* seeds at rate of 1ml of each concentration 1%, 2%, 3% and 4% (Ashamo and Akinnawonu, 2012) was mixed with 20g of clean wheat grains in 250 ml plastic containers. The extracts were thoroughly mixed with the aid of a glass rod and agitated for 5-10 min to ensure uniform coating. The containers were left open for 30 min so as to allow traces of solvent to evaporate off (Ileke and Olotuah, 2012). Two control experiments were set up, one treated with solvent (Arannilewa *et al*., 2006) and another without solvent treatment (Ashamo and Akinnawonu, 2012). Twenty newly emerged adults *R. dominica* were randomly (unsexed) picked and introduced to each of the containers and covered. Four replicates of the treatments and untreated controls were laid out in Complete Randomized Design. Beetle mortality was observed daily for 4 days. After every 24 hours, the number of dead beetles were counted and recorded. The beetles were confirmed dead when there was no response to probing with sharp pin at the abdomen (Adedire *et al*., 2011). At the end of day 4, all insects, both dead and alive were removed from each container. The experiment was kept inside the insect cage for another 30 days to allow for the emergence of the first filial (F_1) generation. The number of adults that emerged from each replicate was counted and recorded. The percentage reduction in adult emergence of F_1 progeny or inhibition rate (IR) was calculated as described by Tapondju *et al*. (2002). The % loss in weight was determined and recorded using the method described by Odeyemi and Daramola, 2000.

2.5. Statistical analysis

Data were subjected to analysis of variance and treatment means were separated using the New Duncan's Multiple Range Test. Data were subjected to analysis of variance (ANOVA), and means were separated using New Duncan's Multiple Range Test. The ANOVA were performed with SPSS 16.0 software (SPSS, Inc., 2007).

3. Results

3.1. Toxicity of plant powders to R. dominica

The effect of *A. indica* and *P. guineense* seeds powders on mortality of lesser grain borer, *R. dominica* is shown in Table 1. All the plants powders at all tested concentration had above 78.8% mortality after 72 hours of post treatment. Neem seed powders caused 49.3%, 77.5%, 100% and 100% mortality of adult *R. dominica* at rates 0.5/20g, 1/20g, 2/20g and 4/20g of wheat grains after 72 hours of application respectively. There was no significant difference (*P*>0.05) in mortality of adult *R. dominica* among the grain treated with *A. indica* and *P. guineense* powders causing 100% mortality at all concentration tested after 96 hours of post treatment.

Table 1. Toxicity of plant powder on *R. dominica*

Plant powder	Conc. g/20g of wheat	Percentage mortality at hours post treatment (%) 24	48	72	96
A .indica	0.5	26.3± 2.4b	49.3± 2.4c	83.3± 3.2b	100.0± 0.0b
	1.0	42.5± 1.4c	77.5± 1.4d	100.0± 0.0c	100.0± 0.0b
	2.0	88.8± 1.3f	100.0± 0.0e	100.0± 0.0c	100.0± 0.0b
	4.0	91.3± 2.4f	100.0± 0.0e	100.0± 0.0c	100.0± 0.0b
P. guineense	0.5	15.0± 4.1b	33.3± 3.2b	78.8± 1.3b	96.3± 1.2b
	1.0	25.0± 4.1b	52.8± 1.3c	88.8± 1.3b	100.0± 0.0b
	2.0	58.8± 1.3d	86.3± 1.2d	100.0± 0.0c	100.0± 0.0b
	4.0	73.3± 3.2e	100.0± 0.0e	100.0± 0.0c	100.0± 0.0b
Control	0.0	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a

Each value is a mean ± standard error of four replicates. Means within the same column Followed by the same letter(s) are not significantly different at *P*>0.05 using New Duncan's Multiple Range Test.

3.2. Effect of plant powders on adult emergence, % reduction in adult emergence of F_1 progeny and weight loss

The two plants powders significantly reduced the number of adult emergence in all the concentration tested (Table 2). Percentage reduction in F_1 progeny increased with increase in concentration of plant powders. Neem powder at rates 2/20g and 4/20g of wheat grains had the reduction of 100% F_1 *R. dominica*. Wheat grains treated with plants powder showed no significant difference (P<0.05) in weight loss caused by *R. dominica*.

Table 2. Effect of plant powder on adult emergence and weight loss of wheat grains.Each value is a mean ± standard error of four replicates. Means within the same column Followed by the same letter(s) are not significantly different at P>0.05 using New Duncan's Multiple Range Test.

Plant powders	Conc. g/20g of wheat	No of adult Emergence	% Reduction in adult emergence	% weight loss
A .indica	0.5	4.0	91.3±2.4b	2.5±3.2a
	1.0	2.0	95.02.0b	1.3±2.4a
	2.0	0.0	100.0±0.0b	0.0±0.0a
	4.0	0.0	100.0±0.0b	0.0±0.0a
P. guineense	0.5	6.0	87.5±4.6b	3.8±1.3a
	1.0	4.0	91 3± 2.4b	2.5±3.2a
	2.0	3.0	93.3± 3.2b	1.3±2.4a
	4.0	0.0	100.0±0.0b	.0±0.0a
Control	0.0	46.0	0.0± 0.0a	83.3±2.4b

3.3. Toxicity of plant extracts to R. dominica

Lesser grain borer mortality in wheat grains treated with *A. indica* and *P. guineense* extracts differed significantly (*P*>0.05) from the two controls experiment (Table 3). The two plants extracts showed that lesser grain borer, *R. dominica* mortality increased with increasing hour of exposure. The two plants oils were effective against *R. dominica* causing 100% mortality at all concentration tested after 48 hours of post treatment.

Table 3. Toxicity of plant extract on *R. dominica.*

Plant extract	Concentration %	Percentage mortality at hours post treatment (%)			
		24	48	72	96
A . indica	1	72.5± 2.4de	100.0± 0.0c	100.0± 0.0c	100.0 ±0.0c
	2	86.3± 1.2ef	100.0± 0.0c	100.0± 0.0c	100.0 ±0.0c
	3	91.3± 2.4f	100.0± 0.0c	100.0± 0.0c	100.0 ±0.0c
	4	100.0± 0.0f	100.0± 0.0c	100.0± 0.0c	100.0 ±0.0c
P. guineense	1	58.8± 5.2c	100.0± 0.0c	100.0± 0.0c	100.0 ±0.0c
	2	62.5± 2.4cd	100.0± 0.0c	100.0± 0.0c	100.0 ±0.0c
	3	76.3± 1.2e	100.0± 0.0c	100.0± 0.0c	100.0 ±0.0c
	4	88.8± 5.2ef	100.0± 0.0c	100.0± 0.0c	100.0 ±0.0c
Control (TS)	0.0	10.0± 4.6b	26.3± 1.2b	38.8± 5.2b	040.0 ±4.6b
Control (WS)	0.0	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0 ±0.0a

Each value is a mean ± standard error of four replicates.
Means within the same column Followed by the same letter(s) are not significantly different at *P*>0.05 using New Duncan's Multiple Range Test.
Keys: TS- Treated with Solvent, WS- Without Solvent

3.4. Effect of plant extracts on adult emergence, % reduction in adult emergence of F_1 progeny and weight loss

The two plant extracts completely had no adult emergence, 100% reduction in F_1 progeny and 0% weight loss (Table 4). It can be seen from the table that the % reduction in adult emergence of *R. dominica* and weight loss in wheat grains were significantly higher (*P*>0.05) in the two controls experiments than treated ones.

Table 4. Effect of plant extract on adult emergence and weight loss of wheat grains.

Plant extract	Concentration %	Percentage mortality at hours post treatment (%)			
		24	48	72	96
A . indica	1	72.5± 2.4de	100.0± 0.0c	100.0± 0.0c	100.0± 0.0c
	2	86.3± 1.2ef	100.0± 0.0c	100.0± 0.0c	100.0± 0.0c
	3	91.3± 2.4f	100.0± 0.0c	100.0± 0.0c	100.0± 0.0c
	4	100.0± 0.0f	100.0± 0.0c	100.0± 0.0c	100.0± 0.0c
P. guineense	1	58.8± 5.2c	100.0± 0.0c	100.0± 0.0c	100.0± 0.0c
	2	62.5± 2.4cd	100.0± 0.0c	100.0± 0.0c	100.0± 0.0c
	3	76.3± 1.2e	100.0± 0.0c	100.0± 0.0c	100.0± 0.0c
	4	88.8± 5.2ef	100.0± 0.0c	100.0± 0.0c	100.0± 0.0c
Control (TS)	0.0	10.0± 4.6b	26.3± 1.2b	38.8± 5.2b	040.0± 4.6b
Control (WS)	0.0	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a

Each value is a mean ± standard error of four replicates. Means within the same column Followed by the same letter(s) are not significantly different at P>0.05 using New Duncan's Multiple Range Test.
Keys: TS- Treated with Solvent, WS- Without Solvent

4. Discussion

The results obtained in this study showed that powders and extracts of *A. indica* and *P. guineense* seeds tested were toxic to the lesser grain borer. *R. dominica* and suppressed their population growth in treated wheat grains.

Neem, *A. indica* seed powder and extract completely killed adult *R. dominica* in stored wheat grains. Some researchers who had earlier evaluated *A. indica* powder and extract as botanical insecticides and grains protectant had found them to be effective against maize weevil, *S. zeamais* and cowpea bruchid, *C. maculatus* (Butterwoth and Morgan, 1968; Jackai and Oyediran, 1991; Onu and Baba, 2003; Ileke and Oni, 2011; Ileke and Bulus, 2012). The toxicity of neem to stored products insects has been attributed by various authors to the presence of many chemical ingredients such as triterpenoids, which includes azadirachtin, salanin, meliantriol (Butterwoth and Morgan, 1968; Mbailao *et al*., 2006; Ileke and Oni, 2011).

Black pepper, *P. guineense* caused 100% mortality of adult *R. dominica* within 4 days of application. The biopesticide activity of *P. guineense* could be attributed to the presence of chavicin and piperine, an unsaturated amide (Lale, 1992). Black pepper has been found to be effective against the adults of yam moth, *Dasyses rugosella* (Ashamo 2004). Ofuya and Dawodu (2002) observed a significant reduction in adult emergence of *C. maculatus* when *P. guineense* seed powder was applied at five rates 0.1, 0.2, 0.3, 0.4 and 0.5/20g of cowpea seeds.

Asawalam and Emosaire, 2006; Ileke, 2008; Ileke and Bulus, 2012 also observed that fine particle sizes of *P. guineense* seed powder completely protected maize, sorghum and cowpea seeds respectively. The lethal effect of these plants powders and extracts could be as a result of contact toxicity (Adedire *et al*., 2011). Most insects breathe by means of trachea which usually leads to the surface of the body called spiracle. These spiracles might have been blocked by the powders and extracts thereby leading to suffocation.

The percentage reduction in adult emergence and no adult emergence observed in all treated seeds with extracts could be as a result of high mortality of adult insects. The extracts inhibit locomotion which disrupt mating and sexual communication as well as deterring females from laying eggs and complete suppression of the developmental stages of insect an effect that had been reported by many researchers (NRC, 1992; Adedire, 2002; Akinkurolere *et al*., 2006; Ileke, 2008; Oni and Ileke, 2008; Akinkurolere *et al*., 2009; Adedire *et al*., 2011; Ileke and Oni, 2011; Ileke and Olotuah, 2012). The lower F_1 adult *R. dominica* in wheat grain treated with *A. indica* and *P. guineense* powders at rates 0.5/20g and 1.0/20g of wheat may be as a result of concentration used. However, the bioactive constituents of the plant materials may be more available in the extract which may be responsible for the higher mortality of adult insect within a very short time of exposure (Ashamo and Akinnawonu, 2012). The results obtained from this study justified that powders and extracts of *A. indica* and *P. guineense* seeds can be used as biopesticides against *R. dominica*. The two plants are of medicinal values, biodegradable, readily available and poses no danger to man and other mammals.

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